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RELATION OF SPECIFIC PRECIPITATION TO OTHER IMMUNITY REACTIONS *

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Within the last few years much interest has been manifested in the balance in the blood stream between the serum protease and its antiferment.

It has previously been supposed that, when protein antigens were injected into the body, specific proteolytic ferments were formed which would split the specific protein into simpler compounds. In the anaphylactic reaction a specific proteolytic ferment was thought to be formed which split the antigen on second injection, into toxic products. In the Abderhalden reaction the dialyzable substances were supposed to be formed by the action of a specific ferment on the substrate. The source of the split products was always believed to be the antigen.

It is easily shown that serum always contains a nonspecific protease that is normally prevented from acting by an antiprotease (antitrypsin). Serum will inhibit the proteolytic power of trypsin. The amounts of protease and antiferment seem to vary greatly under different conditions. Many have believed that the ferment-inhibiting substances of the blood are true antibodies, but very little proof has been advanced to support the view.

Jobling and Petersen[1] noticed that soaps of the unsaturated fatty acids inhibited the action of trypsin. From later experiments[2] they concluded that the ferment-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids. These same investigators showed[3] that sera from which the lipoids had been removed became toxic for the homologous animals. They explained the toxicity as due to exposure of the serum proteins to the action of the serum protease with a consequent formation of toxic split products. They also observed changes in the coagulation of the blood showing that the mechanism of coagulation was altered. They believe therefore that the unsaturated lipoids play an important part in the mechanism of coagulation.

In anaphylactic shock there is no doubt that poisonous protein split products are the etiologic factors. This has been demonstrated in vitro. There is now a consensus of opinion regarding the rôle of protein poisons in the production of anaphylaxis, but there has been a diversity of opinion regarding the matrix, that is, the protein broken down.

Donati[4] concluded that the antigen was not split since organisms so used retained their antigenic properties. Now we find that so-called anaphylatoxin can be produced, in vitro, by treating sera with absorbing substances, such as kaolin, agar, starch, bacteria, etc.

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[1] Jour. Exper. Med., 1914, 19, p. 239.

[2] Ibid., p. 459.

[3] Ibid., p. 480.

[4] Arch. p. le. sc. med., 1912, 26, p. 421.

Jobling and Petersen showed lately[5] that serum antiferment (unsaturated lipoids) could be removed from sera by kaolin, starch, agar, and bacteria, and that sera treated in this way were toxic for the homologous animals. They concluded that anaphylatoxin represented serum rendered toxic by partial removal of the antiferment. They found that bacteria so treated were more resistant to the action of trypsin. From these experiments they placed the matrix of the protein split products on the serum proteins so exposed. They found that sublethal doses of soap solutions injected simultaneously with antigen prevented anaphylactic shock.

Recently Jobling, Petersen, and Eggstein[6] investigated the changes in serum ferments, antiferments, and protein split products during anaphylactic shock. They found that injection of the antigen into a nonsensitized animal was followed by practically no change in the serum ferments or protein split products. Further, that following the injection the body responded by a progressively increasing rapidity of mobilization of nonspecific protease, the amount of protease mobilized becoming greater as the maximum of sensitization was reached. Acute anaphylactic shock was accompanied by an immediate and marked increase in serum protease, by a fall in antiferment, by a rise in the aminoacid content and noncoagulable nitrogen in the serum, together with a primary decrease in serum proteoses. They summarized the mechanism of anaphylactic shock as: "An immediate mobilization of nonspecific protease in large amounts, the formation of antiferment deficiency through colloidal changes, a simultaneous cleavage of serum proteins through the peptone stage to amino acids, an intoxication by these peptones with a resulting cellular injury made evident by an increase in serum lipase, fall in temperature and other manifestations of acute shock. The elements of specificity lie in the mobilization of the nonspecific cellular ferment and the colloidal changes in the serum, not in the production of a specific ferment."

In the last few years much interest has been aroused in the Abderhalden reaction. Since this method has been available many investigators have published results in which the desired specificity was obtained, and others results which tended to discredit the specificity of the reaction. As stated before, it has always been assumed that the antigen itself is split by specific ferments. But specific ferments have never been demonstrated. The serum protease has been shown to be nonspecific. Jobling, Petersen, and Eggstein[7] have shown that the placental cells are not digested during the Abderhalden reaction, and that during the reaction the placental cells become more resistant to ferment action because of the absorption of serum antiferment. They concluded that the substrate digested is the serum proteins made available for protease action by the removal of the antiferment.

Bronfenbrenner[8] found that if placental cells were placed in contact with immune serum at 0 C. for 16 hours and then were removed, the serum was not digested, but that if the serum so treated was then incubated at 37 C., digestion took place. He found that placental cells previously treated with immune sera were capable of removing the antiferments from normal sera by contact at 0 C. Such normal sera when separated from these cells and incubated at 37 C. were digested. He removed normal serum from contact with this sensitized placenta and added fresh portions of normal serum to the same portion of treated placenta ten times, with the result that even the tenth portion of normal serum showed marked autodigestion when incubated at 37 C. This showed undoubtedly that the placenta once sensitized acquired the property of removing the antiferment from the serum. He also found that pregnant serum actually lost its specific substances during contact with placenta at 0 C. Returning the serum antiferment to the serum previously exhausted of its antiferment stopped the autodigestion. He concluded, "the Abderhalden test may be resolved into two phases: First, the sensitizing of the cells so that they attained the property of removing the antiferment, and second, the autodigestion of the serum." Smith[9] obtained similar results using bacterial cells instead of placenta.

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[5] Jour. Exper. Med., 1914, 20, p. 37.

[6] Ibid., 1915, 22, p. 401.

[7] Ibid., 21, p. 339.

[8] Ibid., p. 221.

It seems that in the Abderhalden reaction and the anaphylactic reaction the immune serum causes a change in the antigen, after which the antigen has attained the property of removing the antiferment from the serum. The antiferment being removed the nonspecific protease can digest the serum and thus form the protein split products.

It is known that the immune sera before the anaphylactic shock and the Abderhalden reaction contain precipitins. Also it has been noticed by Joachimoglu[10] that precipitins disappear instantly with anaphylactic shock. It is plausible to suppose that some change similar to a precipitin reaction must take place in these reactions. After observing these facts it occurred to me to ascertain to what extent the precipitate from a precipitin reaction would remove the antiferment from sera.

A rabbit was immunized to extract of fresh beef. After numerous injections the serum showed marked precipitation with beef extract. The rabbit was then bled and the serum obtained. The serum was added to ten times its volume of beef extract diluted 1:1000 and allowed to incubate for 2 hours. It was then placed in the ice box over night. The next day the precipitate was centrifugated from the supernatant liquid and washed four times. The precipitate, which measured 0.02 c.c. in a Hopkins tube after centrifugation for 10 minutes at high speed, was made up to 20 c.c. with normal salt solution. This suspension was used in the following experiments:

Volumes of 1 c.c. each of serum from a rabbit immunized to beef extract were mixed with varying amounts of suspension of precipitate. One tube was left as a control. These were all made up to the same volume and incubated for 3 hours. The total nitrogen and the noncoagulable nitrogen for each tube were determined.

The results of this experiment are shown in Table 1. The increase in noncoagulable nitrogen shows the amount of digestion. The suspension of precipitate contained only traces of noncoagulable nitrogen. The nitrogen was determined by the method of Folin and Denis[11] with slight variations. The coagulable and noncoagulable nitrogen were separated by adding to the tubes an equal volume of a solution of 10% glacial acetic acid and 20% sodium chlorid. The tubes were then boiled 5 or 10 minutes and the contents filtered through hard filter paper impregnated with kaolin.

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[9] Jour. Infect. Dis., 1916, 18, p. 14.

[10] Ztschr. f. Immunitäts., 1911, 8, p. 453.

[11] Jour. Biol. Chem., 1911-12, 11, p. 527.

TABLE 1

Results of Digestion in Rabbit Immune Serum and Specific Precipitate

Tube	Precipitin Rabbit Serum, c.c.	Suspension of Precipitate, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen, mg.
1	1	0.8		0.31
2	1	0.4		0.35
3	1	0.2		0.43
4	1	0.1		0.36
5	1	0		0.07
6	1	0	2.01	

This experiment was now repeated numerous times with normal rabbit serum. Marked digestion was noted similar to that in immune serum. The results in one case are shown in Table 2. It was noted that a certain amount of the suspension is the most active; more than this amount or less does not produce as much digestion. This will be discussed later. From this experiment it seems that the precipitate will remove the antiferment from the normal serum.

TABLE 2

Results of Digestion in Normal Rabbit Serum and Specific Precipitate

Tube	Normal Rabbit Serum, c.c.	Suspension of Precipitate, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen, mg.
1	1	1		0.54
2	1	0.5		0.64
3	1	0.1		0.66
4	1	0.01		0.51
5	1	0		0.28
6	1	0	1.84	

Since Bronfenbrenner and Smith found that cells sensitized with their immune serum would remove the antiferment from all sera from the same species from which the immune serum had been obtained, and since the precipitate from a precipitin reaction removes the antiferment from the homologous normal serum, I tried to determine whether this reaction was specific for the homologous serum or not. I endeavored to find whether these substances would remove antiferment from other sera.

Colon bacilli were treated with serum from a rabbit immunized against B. coli. A 24-hour culture of B. coli was suspended in 3 c.c. of normal salt solution mixed with 3 c.c. of immune rabbit serum, and incubated for

2 hours. The organisms were then removed by centrifugation and washed four times. The bacilli, which now measured 0.05 c.c. in a Hopkins tube, were made up to 25 c.c. with normal salt solution. They were then used in the following experiments. The suspension contained only a trace of noncoagulable nitrogen. Experiments showed that in small quantities it caused digestion in normal rabbit serum. Jobling showed that normal bacteria in large amounts removed the antiferment from serum, but the removal would at least be small with such small quantities of normal bacteria.

Normal horse serum, sheep serum, and guinea-pig serum were treated with different amounts of sensitized colon bacilli and precipitate, both from rabbit serum. These were incubated 3 hours and then the total nitrogen and the noncoagulable nitrogen were determined, also the noncoagulable nitrogen in the controls. The results are shown in Tables 3, 4, and 5. It will be seen that there was a marked increase in the noncoagulable nitrogen in the sera so treated. All the experiments were repeated several times with similar results.

TABLE 3

RESULTS OF DIGESTION IN NORMAL HORSE SERUM AND SENSITIZED COLON BACILLI

Tube	Normal Horse Serum, c.c.	Suspension of Precipitate, c.c.	Suspension of B. Coli, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen in 1 c.c., mg.
1	3	0.5			0.60
2	3	0.1			0.62,
3	3	0.01			0.58
4	3	0.001			0.46
5	3		1		0.56
6	3		0.5		0.58
7	3		0.1		0.62
8	3		0.01		0.65
9	3		0.001		0.39
10	1	0	0		0.40
11	1	0	0	2.12	

TABLE 4

RESULTS OF DIGESTION IN SHEEP SERUM AND SENSITIZED COLON BACILLI

Tube	Sheep Serum, c.c.	Suspension of Precipitate, c.c.	Suspension of Sensitized B. Coli, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen in 1 c.c., mg.
1	2	0.5			0.42
2	2	0.1			0.43
3	2	0.01			0.39
4	2		0.5		0.41
5	2		0.3		0.42
6	2		0.1		0.40
7	2		0.01		0.34
8	2		0.001		0.21
9	1	0	0		0.24
10	1	0	0	1.98	

TABLE 5

RESULTS OF DIGESTION IN GUINEA-PIG SERUM CAUSED BY PRECIPITATE AND COLON BACILLI FROM RABBIT SERUM

Tube	Guinea-Pig Serum, c.c.	Suspension of Precipitate, c.c.	Suspension of Sensitized B. Coli, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen, mg.
1	1	1			0.50
2	1	0.1			0.52
3	1	0.01			0.49
4	1		1		0.49
5	1		0.3		0.51
6	1		0.01		0.34
7	1	0	0		0.41
8	1	0	0	2.02	

Since we should expect a similar and probably more pronounced action in pathologic sera, the serum of a tuberculous guinea-pig was treated in a similar way with sensitized colon bacilli. There was a marked increase in digestion, as shown in Table 6.

TABLE 6

RESULTS OF DIGESTION IN SERUM FROM A TUBERCULOUS GUINEA-PIG AND SENSITIZED COLON BACILLI

Tube	Tuberculous Guinea-Pig Serum, c.c.	Suspension of Sensitized B. Coli, c.c.	Total Nitrogen, mg.	Noncoagulable Nitrogen, mg.
1	1	1		0.55
2	1	0.1		0.56
3	1	0.01		0.43
4	1	0		0.33
5	1	0	2.08	

DISCUSSION

These experiments show that the precipitate from a precipitin reaction will cause digestion in normal serum, and that this takes place with very small amounts of precipitate. Digestion is probably due to the removal of antiferments, allowing the nonspecific protease to act on the serum proteins. This seems to show the possible relationship of precipitins to other immunity reactions. Precipitins have been thought by many to be concerned in the anaphylactic reaction, some having thought precipitin and anaphylactin to be identical. Precipitin disappears after anaphylactic shock and passive anaphylaxis is conferred in proportion to the concentration of precipitin in the serum.

In the precipitin and anaphylactic reactions the serum has developed the property of reacting with the specific antigen with the result that a new body is formed in the serum. These experiments seem to show that this body now possesses the property of removing the antiprotease from the serum, or, rather, of rendering it inactive. Therefore, it seems possible that in anaphylactic shocks the serum proteins are exposed in this way to the action of nonspecific protease with toxic split products as the result. It seems that the mechanism of the Abderhalden reaction must be identical with the reaction mentioned. The immune serum has the same specific action on the surface of the cells, after which they possess the property of removing the antiprotease from the serum. After this removal the dialyzable peptones and amino-acids are formed by the action of the nonspecific protease on the serum proteins. Thus we see that the so-called precipitins may be concerned in the anaphylactic and Abderhalden reactions. In other words, it may be the precipitins which react with the antigens, thus forming new bodies which remove the antiprotease.

Bronfenbrenner has previously shown that the removal of antiprotease by sensitized cells is nonspecific in the homologous sera. My experiments seem to show that sensitized cells, and also precipitate formed by the action of immune serum and antigen, will not only remove the antiprotease from the homologous sera, but also from heterologous sera (horse, sheep, and guinea-pig). It therefore seems that this is a nonspecific reaction.

It is not clear just how the unsaturated lipoids are rendered inactive. One way might be by the saturation of the unsaturated bonds, but this is not likely. Again it may be by absorption of the lipoids, since the cells so treated become more resistant to the action of trypsin. This may take place with large amounts of kaolin, bacteria, etc., but it hardly explains the action of small amounts of precipitate and sensitized cells. A third method may be that of changing the state of dispersion of the lipoids. The unsaturated lipoids are inactive as ferment-inhibitors unless in a highly dispersed state. The action of precipitate and sensitized cells is probably that of changing the state of dispersion of the unsaturated lipoids so as to render them inactive.

It will be seen that the specificity in these reactions lies in the action of the immune serum on the antigen (a physical change). After this has taken place the removal of the protease-inhibitors (a physical change) and also the action of the serum protease (chemical change) are nonspecific.

It will be noticed that maximal digestion occurs with certain quantities of precipitate or sensitized organisms. Larger or smaller quantities cause less digestion. Jobling called attention to the fact that there is a quantitative relation between the amount of kaolin and the maximal absorption of antiferment.

It will be noticed that sometimes with very small amounts of precipitate or sensitized organisms less digestion resulted than in the controls. Jobling also noticed similar results. It is suggested that in the case of bacteria this might be explained by the fact that the very small amounts may be dissolved and thus liberate the antiferment absorbed and also their own ferment inhibitors.

CONCLUSIONS

In small amounts the precipitate formed by action of immune serum and antigen, and also sensitized organisms, will remove antiferments from sera. This may show the relation of precipitins to anaphylactic and Abderhalden reactions.

The absorbing action of sensitized organisms and precipitate is not specific. They will remove the antiferment not only from the homologous sera, but also from heterologous sera.

There is a quantitative relation between the amounts of precipitate and sensitized organisms and the maximal amount of absorption of the antiferment.